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Moderator: Heather Doyle
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Heather Doyle: Hello everyone. As she said, I'm Heather Doyle, from the Solar System Ambassador's Program, and on behalf of Kay Ferrari, who's also from the Solar System Ambassador's Program, and Jeff Nee from the Museum Alliance, I'd like to welcome you all to our telecon. And thank you for joining us.

So today, we'll be talking about Nanopore Sequencing in Space: Applications for Crew Health, Research, and Astrobiology. It seems this will be increasingly important as we look to sending people to Mars, I would assume. But we're privileged to hear from Dr. Aaron Burton. Dr. Burton is an Organic Geochemist and Astrobiologist at NASA Johnson Space Center. His main areas of research is focused characterizing and understanding the origins of soluble, organic matter in meteorites and investigating the role these compounds might've played in the origins of life on Earth and elsewhere in the universe.

So, Dr. Burton, without any further ado, I turn it over to you.

Dr. Aaron Burton: Thank you for the introduction and for the opportunity to give this presentation today.

So this is a little bit outside of my normal day job, this project with the sequencer, but goes closer to what I did as a graduate student, where I worked on ribonucleic acid -- or RNA -- enzymes with an eye towards understanding how RNA could've contributed to the origins of life on earth.

So first slide, [Slide 2]. So just a little overview of my talk today. So we're going to talk about nanopore sequencing as a life detection tool. At this stage, we don't have a lot of results, so we're kind of in the formulation stage, trying to get money sort of a thing. But so just kind of some conceptual applications of nanopore sequencing as a life detection tool.

Then some recent experiments, we were able to do on the International Space Station with an eye towards using the nanopore sequencing to sort of help further human exploration into the solar system. So that's kind of a general idea of what we're going to talk about.

And so one of the key questions for me that got me excited about doing research, and is probably the reason I ended-up at NASA -- was trying to understand how life started on Earth. And so on the next slide – [Slide 3], -- there's just a timeline for the origins of life on Earth.

We know that the Earth formed a little over four and a half billion years ago. We got water around 4.2 billion years ago. And then we had some sort of magical time where you had prebiotic chemistry going on. And then we fast forward to, say, 3.8 to 3.6 billion years ago where we have kind of the first fossil evidence of life.

And then on to the great evolutionary diversity that we have today. We kind of understand the very beginning of that timeline with the Earth forming and water from geologic samples. And then we know a reasonable amount from where we have sort of the first fossilized life forms, about 3.6 billion years ago. But in that middle part we don't really have a lot of good records there.

And so, you know, a key area of astrobiology research is understanding how life started on Earth and how or whether those processes could've occurred in other places.

I've drawn a red box in here around time points that are called sort of the pre-RNA world, the RNA world and the first DNA protein life. So one of the most widely accepted hypotheses for the origins of life is the so-called RNA world hypothesis. And so, that's what we'll talk about for a little bit here.

So, if you go to the next slide, Number 4 -- I apologize for not putting slide numbers on these -- we have the RNA world hypothesis, which has been described in a number of ways, but was originally attributed to Wally Gilbert in 1986. It is founded on the premise that in current biology, we know the RNA serves and informational role. So for example, messenger RNAs contain the actual code that tells the ribosome what amino acids to put into a protein. So, RNA is carrying the information from DNA to the ribosome so that we can make proteins.

We also know that RNA performs catalytic functions in contemporary biology. So this was first discovered by (Tom Cech) and (Sid Altman) in the late-70s - early-80s, where they found RNA enzymes that were capable of splicing other RNA molecules. And so these are processes that are actually happening in current biology now.

And so when you look at those two facts together, we know the RNA can do the job of proteins, and we know that RNA can do the job of DNA, but it happens to be less stable than DNA and it is not as good of a catalyst as proteins. And so from a logical approach, it makes sense that maybe RNA was there before we had DNA and before we had proteins, and then DNA evolved as a more stable informational macromolecule and proteins evolved to give us

better catalysis. Because it wouldn't make sense if you already had DNA to evolve RNA, which is less stable. If you already had good system of protein catalyst, why would you evolve RNA with catalytic functions?

So this is the prevailing paradigm in astrobiology for how life started on Earth.

So one of the biggest knocks against it – if we go to the next slide, [Slide 5] – is that RNA actually is pretty complicated.

So if you look over in the far right hand side of the screen, there's a little pentagon, and there's a two prime, a three prime, and a five prime. So this is ribose -- or deoxyribose -- sugar. And it's got this pentagon structure. Next to that two prime, you've got an H that has the dashes and in RNA that would actually be an OH. And then if we move to the left -- at the three prime positions -- we've got a H that's a solid wedge and then dashed wedges. And then if we go up and to the left, there's another Carbon below the five prime that has the dashes to the H.

And so for those of you that aren't necessarily chemists, those dashes are supposed to tell you something about the stereochemistry of that molecule.

So at the two prime position that hydrogen is actually facing into the screen. And then at the three-prime position, that bond down to the O -- or oxygen -- is also facing into the screen. And then the solid wedge to the hydrogen on that three prime that's actually poking out should be sticking out of the screen, like pointing towards us. And then up one more carbon where that H is, that's also sticking into the play on the screen.

And so at each of those places, those are called chiral centers. And so there's two different stereo chemical ways that you can arrange those.

So for ribose or this five carbon sugar there's two possibilities at two prime, two possibilities at three prime, and two possibilities at four prime. So there's eight different ways just with all the same atoms you could make this molecule.

And so it's kind of unlikely in a prebiotic chemistry scenario that you would actually get all those to be in the right configuration.

So up and to the right of that two prime position, there's the B, so that would be the base -- so, like, adenine, guanine, cytosine, and thymidine, or the AGCT of DNA.

Then we also have sort of a phosphate group down from that three prime carbon that connects two different monomer nucleotides together.

So, there's kind of a lot going on here, and people have said, "Well how does that arise from just simple prebiotic chemistry if you didn't have anything before it?" So people have proposed sort of simpler precursors.

To the left of that we have FNA, for flexible nucleic acid, and then TNA, or threose nucleic acid. So in this case, that ribose sugar has been replaced with threose, which as one less carbon and one less chiral center. So it should be theoretically simpler. Now there's only four possibilities instead of the eight that I mentioned stereochemically.

And then even simpler than that, to the left we have GNA, or glycerol nucleic acid.

So people have proposed these as sort of RNA precursors that maybe could've eventually led to the evolution of RNA for the RNA world hypothesis.

Next slide- [Slide 6]. So, it's great that people have proposed these, and some early experiments show that you could make the double-stranded duplex, like we see in DNA, molecules containing some of these other sugars. But you also need to show that you can do something with them.

And so in some very nice experiments, the Chaput and Holliger Labs, in 2012, show that threose nucleic acid, which is -- in the panel -- Figure D, and then hexose nucleic acid, which is not shown here, they show that they could actually do evolution experiments in a test tube and develop enzymes out of these sugars. So, instead of the ribozymes, they would have a threosezyme.

And so these were really important experiments to show that some of these alternatives -- nucleic acids or xeno nucleic acids, XNAs could actually do the same job as RNA in that they can carry information and they can also do a function like a protein would.

The XNAs are capable of doing the job of RNA and so maybe some of them like threose nucleic acid are simpler, so maybe that's where it started from.

Next slide, [Slide 7]. So those are some possible alternatives to DNA and RNA in sort of a origins of life scenario. But we even know that in contemporary biology, on Earth, we don't just have standard DNA and RNA with the AGCT or U bases. Because we know that we have the DNA bases getting methylated so epigenetics, we know that genes get turned on and turned off at different

points in time for an organism to regulate growth and other things. There's at least one virus that uses 2,6- diaminopurine, which is molecule the lower left hand corner. So that's an analog of adenine, but in this virus, it doesn't have adenine at all. It only has this 2,6- diaminopurine.

We also know that in biology on Earth, the RNA is modified very heavily, so 112 base modifications have been identified in RNA, and mostly in the ribosomal RNA, but in other molecules. So, again, you're not just looking for the standard AGC and U in RNA in this case. But you need to be able to look for a whole range of other molecules.

And then we also know that in proteins, everybody is taught that there's a standard alphabet of 20 amino acids that all of biology uses, but some organisms actually use more and different amino acids. So in the middle image on this page, there's the amino acids selenocysteine, which is like cysteine but the SH group on cysteine – or the sulfur – has been replaced with the selenium atoms. And so that's just a different variant.

And then over on the image on the right-hand side shows a standard GC base pair. And then on the image below it there's this LA base pair. And so L is actually lysidine. So this used to be a cytosine base in a TRNA, and then the amino acid lysine was attached at sort of that bottom position. And so this is used actually in TRNAs when they bind to MRNAs, and it's to make sure that you don't get any wobbling so that the correct amino acid gets put into proteins.

So all of this is to say that if we're going to search for life elsewhere in the solar system, I think it would be a mistake to look just for life exactly as we know it on Earth because life exactly as we know it on Earth actually has

some pretty weird things in it, like this lysidine or selenocysteine or all of the different RNA base modifications.

Then there's no guarantee that life elsewhere -- say on Mars or on an ocean world -- would use exactly the same sugar or bases as we use on Earth, maybe not even phosphates. So we need to have a more open approach when we search for life beyond Earth.

[Slide 8] So that's where, for me, I get excited about the nanopore sequencing technologies that are coming online today. So if we go to the next slide, nanopores -- I think -- are suited for in situ Astrobiology because they've been shown to be able to detect a broad range of charged molecules, and many of the biomolecules – at least in life on Earth – contain charges So nucleic acids or polyanions, they have a phosphate group after every monomer. Proteins often have a charge. They have ionizable side chain groups so... Carboxylic acids or amines, that can either be positively or negatively charged. Most lipids are fatty acids that have a charge on them, with the carboxylic acid group. Then there's a whole host of small molecules that often have charges on them. A lot of that is driven because our medium for life on Earth is water, and the best way to make something soluble in water is to put a charge on it.

So another good sort of inherent property of nanopore sensing is that their inherently small. That's the "nano" in "nanopore", is that there are nanometer scale devices. You can start with the small instruments and not have to build-up a whole lot of infrastructure around it to accommodate it.

Then for the minion DNA sequence, which is what we'll be talking about for most of this. The sample preparation is faster and simpler than other commercially available sequencing platforms.

And so on the bottom of this page there's a picture of a nanopore with a – there's a double strand of DNA, and one strand is sort of going off to the left, and the other strand is being fed through this pore. Then there's a little gray box, and so in that box there's actually a gif or whatever in a pdf file. So you can see it's sort of moving in time, but you have these peaks and valleys on that landscape, and every peak and valley on there, tells you something about the base that's passing through the pore at that time.

So that's kind of how the sequencer works; we'll come back to that in a minute.

I forgot to mention, so if there's any questions, feel free to chime-in and ask.

But so if we go to the next slide, [Slide 9]...

So the versatility of nanopore sensing – we have in this Figure A, there's a current versus time profile. So these are nanopores that have been embedded in membranes that you could see. In that top pore, where there's nothing blocking the membrane, you have a high current flowing through it -- say, like, 115 picoamps. That tells you that nothing is blocking the pore.

In the bottom pore, you have a molecule that's passing through that pore, and then the current is reduced through that pore. In this case, that change in current between the open pore and the blocked pore tells you something about the molecule that's passing through it. Depending on how you design the pore and the platform, you can actually get better or worse base resolution.

And so the extreme example is that DNA sequencing that we're going to talk about, where you can actually get the individual bases that are coming out -- so, the As, the Gs, the Cs, and the Ts.

And so with this nanopore sensing, it's possible to analyze any polymer that actually obstructs the pore. It's currently being applied to DNA and direct RNA sequencing, and people are working on protein analysis as well. So people have looked at the folding and shape of proteins; now it's being modified or developed for those purposes too.

And then to get base-by-base or monomer resolution, you need to change the speed at which the polymer passes through the pore and you can do that in a variety of ways. You can put something on the edge of your polymer to slow it down going through the pore, or you can change the voltages, which will change the rate at which it passes through the pore.

So that's kind of the nuts and bolts of how nanopore sequencing works.

[Slide 10]- And so from this paradigm of RNA, DNA, and then maybe some of these other weird NAs -- the XNAs -- nanopore sequencing has already been shown to not be limited to sort of canonical AGCT DNA.

I mentioned RNA sequencing that's been done. And so usually in the lab if you're going do RNA sequencing you actually do what's called reverse transcription. And so you make a DNA copy of the RNA, and then you sequence that DNA. But with the nanopore sequencing – in particular the MinION, you could actually do direct RNA sequencing.

Chris Carr's lab up at MIT shows that you could sequence inosine containing DNA molecules, and so inosine's shown on the lower right hand corner, this is a guanine analog -- or a G analog -- but the sequencer works just fine with it. If you look to the left, they made a synthetic DNA molecule that contains deoxyinosine and deoxycytosine.

If you look at the current plot – so there's that 5, 0, -5, and then the timeout's 20 seconds. There's two different current states in there, which are- one is the inosine and one is the cytosine.

That was really exciting to me to see that others are kind of working on this idea of looking for sort of non-standard DNA and RNA.

It's also been shown recently that you can do the epigenetic sequencing with the detection of methylated bases. And so there you're looking for genes that are being turned-on or being turned-off. One of the big recent announcements out of the twin study with Scott Kelly's work was that they found that during space flight on his time on the ISS, there was actually a huge increase in the amount of methylation that was going on with Scott Kelly's DNA, and so that's really exciting to be able to detect that directly.

Again, with most sequencing platforms, you would have to actually copy the DNA. During the process of copying the DNA, you actually lose where those methylations are, and so this is kind of a cool technology to be able to have.

And then even a decade ago, nanopores were used to characterize proteins with that folding.

So if we go the next slide – [Slide11], I know there's been a lot more proposals and teams and groups talking about sending a sequencer to Mars. I feel like we're on the cusp where that sort of thing is going to happen, but there's still some things that need to happen. You have to evaluate the stability for a long-duration mission.

So normally -- particularly with the MinION -- you're supposed to use the flow cells within about eight weeks of their manufacture; people have used them out to six months and beyond.

I think that Mars is actually within reach, so you could put these on a Mars rover if you had a really, quick flight. I've heard that it's possible to make it to make to Mars within maybe four months. You could potentially send a sequencer with the technology the way it currently is to Mars, but if you wanted to go to one of the ocean worlds, like Europa where you're looking at multiple years of travel and very high radiation environments, there's going to be more technological development that has to be done.

We also need to really focus and expand the analytical approaches to analyze a broader range of DNA, RNA, and protein-like molecules. So as a nice demonstration by the Carr Group that you could do cytosine and inosine, but we need to look at a whole range of these xeno nucleic acid -- or XNA -- sort of candidate molecules.

And so I'm part of a team that's got a proposal to do this. And I know that there are other groups that are working on this as well.

And so that's sort of the end. This is kind of the theoretical approach to the origins of life. And then the remainder of the talk will be about the

experiments we did on the International Space Station. So this might be a good time if anyone's got some questions to sort of address those before we move-on.

Man 1: Okay. Have amino acids and nucleic acids been found in the Allende CB3 Meteorite? I know they've been found in Murchison and Murray and CM meteorites. Have they been found in the Allende?

Dr. Aaron Burton: So, to my knowledge, we looked at Allende. I was a post-doc at Goddard. We looked at Allende and it seemed like the cleanest samples we could get still had a lot of contamination in them. So we had a paper that came out in 2011, I guess -- or 2012 -- where we looked at those and we thought maybe we found some amino acids that were indigenous but nothing that we could get really, really excited about.

And I think you would run into the same issues. We didn't look for, like, nuva bases or DNA at that time, but I think you would run into the same issues in terms of contamination.

Man 1: Why would Allende be contaminated? It was a fall.

Dr. Aaron Burton: Yes, but it's all in how it's handled when it, you know, after the fall. So, like, Sutter's Mill, for example, it was recovered in California. And the people that were picking it up were picking it up with their bare hands. And so, ideally you would get people out there with gloves and they they'd have, like, sterilized aluminum foil and collect it directly.

Man 1: But if you have a stone, then you go deep enough into the stone it should be pretty pristine, right?

Dr. Aaron Burton: Yes. I'm not saying it's not possible, I'm just saying from the samples that I've been able to analyze we weren't able to get anything. But yes, – there's just so much material you would think that we'd be able to get a clean sample.

Man 1: Right. I'm in an avid meteorite collector, so I have Allende, Murchison, and Murray in my collection.

Dr. Aaron Burton: Okay.

Man 1: So I really love those meteorites.

Dr. Aaron Burton: Yes. Definitely.

Man 2: Dr. Burton, you guys have any plans for the sample return mission from the OSIRIS REx when it comes back from Bennu with some CM material. Do you think – or at least C material, it may not be CM. Is there plans to look in there for organic matter?

Dr. Aaron Burton: Yes, so I know Jason Dworkin at Goddard is the project scientist, and that was the lab I did a Post-Doc in, and I know that their immediate plans during the preliminary examination are going to be to look at amino acids and other organics. I assume that they're going to look for at least the nucleobases of DNA. Then maybe someone like George Cooper at Ames would be able to look at sugars because he already has the techniques in place.

I'm sure that there is a plan to look at all of those different molecules for sure.

Man 2: Thank you.

Heather Doyle: I was going to ask how you create the nanopores. Is it something that's naturally occurring that you repurpose or do you create it from scratch?

Dr. Aaron Burton: Yes. So in the MinION, they're naturally occurring proteins. So normally, in biology you have a lipid bilayer membrane that doesn't allow charged or polar things to pass through. You have to put pores in there that will allow ions and polar compounds to flow in and out. These pores – that are in the sequencer -- are started as an existing ion channel basically. Then they've been modified extensively so a number of mutations in the pore to make it so that it better interacts with the DNA or RNA that's passing through it to make it basically to improve that base resolution as it's going through it.

Especially when you think of going to Europa, people are moving toward solid state nanopores, which are like if you have a silicon wafer you can actually make with an ion beam these ion pores in it, and then...

So that has the advantage that you can kind of tailor exactly the size of the pore that you want. You can look for different things. And those, in principle, are thin enough that you could actually sequence single bases like, RNA or DNA.

The challenge is slowing molecules down. It turns-out, if you apply an electric field then the molecules want to go through really fast, you have to find a way to kind of impede them so that your electronics can measure the signal changes on the same time scale that the molecules are translocating.

Does that make sense?

Heather Doyle: Yes. Thank you.

Jeff Nee: And I had a follow-up question to that. So each base gives a different current reading once it passes through the pore. Is that right?

Dr. Aaron Burton: Yes. And that was a little bit of a simplification. So the pores actually – depending on the pore it'll be, like, three to six bases that actually fit in the pore. You're really measuring say three or six at a time. Then you step forward one base. So then there's five previous bases, plus one new one. And then you step it again. Then there's four of that first set and two new ones, and so you have to do some deconvolution to differentiate all these basically- it's sixmers or threemers. They call them (K-mers).

Does that make sense?

Jeff Nee: Yes. A little bit. So when you get the signal, you still have to deconstruct it and get the actual sequencing out.

Dr. Aaron Burton: Yes.

Jeff Nee: Okay.

Dr. Aaron Burton: Yes.

Jeff Nee: Okay. All right. That makes sense. Okay. Cool. Oh. I had a quick question about the twin study that you mentioned. Was the extra methylation – do they think it's all from the extra radiation that you get up in space or is it something else?

Dr. Aaron Burton: So that's where it's not clear and I don't know enough about that. I just read that NASA press release, so I'll have to dig more into that. But I can imagine a lot of things depending on which genes are being turned on and off.

So maybe... We know that bone loss is an issue, and so maybe because there isn't as big of a load on the the skeletal system maybe the body says, "Well, let's quit making all these bone strengthening proteins because we're just wasting our energy." So there's definitely more to be learned there.

Jeff Nee: Okay. Great. Thanks.

Dr. Aaron Burton: All right. That was great questions.

All right. So the black part of the talk I guess. I got these slides from my co-investigator, Sarah Wallace. She's a microbiologist here at The Johnson Space Center. And do I'm the astrobiology and chemistry part of implementing the sequencer on the ISS and in space, and then she's operationally involved in maintaining cleanliness and crew health on the ISS. And so it's really a nice sort of collaboration and intersection between human exploration and science and astrobiology. So this is pretty fun.

So if you go to the next slide [Slide 13], we wanted to put a DNA sequencer in space. You know, of course anytime you want to do something the question is: "Why?"

So as I mentioned, Sarah is a microbiologist here. Currently on the ISS, to monitor the environment, they actually will swab a sample or collect some water and then grow up the samples on a bacterial or culture plate. Then they return those samples to Earth whenever a SpaceX capsule comes

back down. And then they analyze those samples and they say, “Oh. This is what was in the water you were drinking six weeks ago.”

We’d like to be able to get a better ability to do those assessments in real time to know if there is an issue.

Also, if a crew member shows signs of an infection, right now they talk with their flight surgeon and diagnose whatever’s going on as best they can, but if you had some sort of a infection that went on then you couldn’t really do any precise diagnosis.

We would also like to be able to reduce down mass, so if you can analyze samples inflight -- say on the ISS -- then you don’t have to bring samples back. Or you may not have to.

And of course, all of these issues are magnified as we start thinking about exploration beyond the ISS. If people are really going to go on a 500- or 1,000-day mission to Mars, you’re not going to be returning samples every month so you can do your monitoring. So being able to do those processes in situ is going to make a really big difference; it’s actually going to be essential.

Continuing in the ISS paradigm, we think having a DNA sequencer will be a powerful research tool. We can look at the effects of safe flight on humans while they’re actually inflight so you can get a lot more samples, you don’t have to worry about freezing them with animals – so they have the mice and rats on the ISS, and so it opens up new lines of research that you could do.

And then microbes and cell lines – so that’s the picture sort of in the middle. That’s Staph aureus. So it’s been shown that microbes that are grown in

microgravity change their gene expression. And so something like Staph aureus that's normally gold -- as shown in this control in the middle -- if it's grown under simulated microgravity conditions...

So they have these – the middle picture is this bioreactor that basically spins all the time, so the cells end-up in perpetual freefall, which simulates microgravity. It was found that they don't produce their pigments anymore. It's also been shown that some micro-organisms show increased virulence and crew members showed decreased immune system performance. So it becomes down-regulated.

All of these factors working together, makes it important that we can try to understand the molecular basis for that, why the microbes are getting potentially more dangerous or less dangerous, but also why the astronauts' immune systems aren't responding as well as they should.

That kind of leads into medical operations where if you're getting a crew member medicine or drugs to, for example, prevent bone loss or to keep their immune system active, having a sequencer to be able to look at RNA for gene expression would allow you to actually monitor whether that's working the way that you think it is. You could also look for effects of radiation damage and being able to do real time analysis, especially on a long-duration space mission, could help influence medical intervention.

Then we already talked a lot about the astrobiology, so we don't need to go more into that.

Next slide [Slide 14].

So 2016, was a big year for molecular biology in space. On April 19, the first molecular biology assay in space was completed. That was polymerase chain reaction, or PCR amplification of DNA, using the mini-PCR. The picture to the left, that's a very small device, it's much smaller than the computer it's sitting in front of in the picture to the right.

That was the first time that any molecular biology experiment had been done on the ISS.

Then about ten days later, RNA was isolated, reverse transcribed to DNA, and then the DNA was amplified by quantitative PCR -- or QPCR -- on the ISS using the Wet Lab II Platform. These were two very powerful demonstrations.

In 2016, we also sent the MinION DNA sequencer up to the ISS. It was the biomolecule sequencer payload. This was a technology demonstration. We wanted to evaluate whether just the commercial, off-the-shelf hardware could survive, launched up to space and if it could be successfully operated in microgravity.

[Slide 15] This sequencer, if you haven't seen it, is really small. It is the size of a Snickers candy bar probably a little smaller -- maybe two snack-sized ones put together since it was Halloween yesterday. It's very small, it's very lightweight. There's no battery in it; it's powered entirely by USV connection, which also does the data transfer.

So we launched this on July 18 of 2016 on SpaceX 9.

On the next [Slide 16] shows our whole payload. We had a Surface Pro 3 tablet computer that is basically powering the sequencer and doing the data

collection; we couldn't send the keyboard because it's very flammable apparently. If you light it on fire, it will burn, so don't take your Surface Pro 3 keyboard and light it on fire.

You can see the Surface Pro 3. Then we got the USB cable to the MinION, and then there's a couple of syringes in the bottom of that picture. One of them is to remove an air bubble, and the other contained our sample, and it was frozen in it. Then that round -- or cylindrical -- tube to the next of it just held those syringes.

Stepping to the right, now we've got a picture of the MinION that's open. There's the little black and gray cartridge with a yellow window. Where that yellow window is, is where the nanopores are. There's 2,048 of these little, tiny nanopores that the DNA passes through. There's another sort of schematic of performing the sequencing by looking for current changes over time, then to the right is just the nanopore again.

We sent-up samples that we had prepared on the ground, and they contain the mixture of genomic DNA. The entire genome of lambda bacteriophage, which is the Oxford nanopore technologies who makes the MinION. It's their sort of standard, go-to sample.

We also put in E. coli, and then mouse DNA. We wanted to show that you can sequence – even in the same sample -- DNA from something like a virus or bacteria all the way to a higher organism like a mouse.

We got these libraries ready for sequencing; samples that we prepared for flight, and another set to be analyzed on the ground on the same days, and in parallel with the flight samples.

Dr. Aaron Burton: All right [Slide 17]. The experiment we did for biomolecule sequencer was basically just testing the functionality of the sequencer. I told you about the samples already. You've seen the payload.

On the next [Slide 18], biomolecule sequencer and the data.

In the left-hand picture, that's Astronaut Kate Rubins on the ISS on August 26, 2016. She thawed one of those frozen samples that we had prepared, loaded it in the sequencer -ran it. So within about 20 minutes, we knew that the sequencer was working and that there were no issues. This was a really exciting day for us. It was my first flight payload, and we had no idea if it was going to work, or not, if the nanopores would survive a flight up there. It was a pretty fun day.

So we knew that it had worked.

Next slide [Slide 19].

We ended-up getting nine experiments in total done. We had sent up three that were going to be run with allocated crew time, and then we sent another six that could be done at the crew discretion on the task list.

In total of those nine experiments, they spanned about six months from the time the flow cells were manufactured. In July of 2016 to January of 2017, Kate Rubins did the first seven experiments. The October 25 sample and the 26 sample, was the same sequencing flow cell. And so Kate loaded a sample, started the run, came back, took the flowso out, put it back in the fridge, got it the next day, it came out, and loaded another sample on it to demonstrate that we could actually reuse them.

Then with the November and January samples, that was Peggy Whitson. So it was nice to see that it wasn't just one crew member who could do this, we had nine really successful experiments.

Then if we look at the data on the right-hand side... so Panel A is kind of a summary of all of the data that we got inflight. We put an equal amount of virus, bacteria, and mouse DNA into our samples. We got basically an equal number out, it was about 30% of each were in our read, so that was pretty good. Then we had about 10% of the reads that didn't match up to any of those organisms, which is pretty normal. The nanopore sequencing isn't as accurate as a lot of the standard platforms on Earth, but it has different positives and negatives. One of the biggest positives is the other sequencers weigh, like, 100 pounds or more; they're not very amenable to flying into space and they have complicated fluidics.

Dr. Aaron Burton: Panel D of this is a comparison of the flight and ground data, so the amount that are mapped of the lambda bacteriophage or E. coli or the mouse, and then unmapped. This one sample, but analyzed across the data, everything basically looks the same -- maybe a little bit better in space -- but essentially no difference.

Then Panel C just shows per base accuracy. The one on the left is grounds, and the one in the right is flight; basically they look almost identical.

Man 1: You get a slightly greater peak on the flight sample, a little bit narrower. Is that an artifact of the environment or is that just a normal distribution range?

Dr. Aaron Burton: You know, so it's kind of an interesting thing. For every sample we did some of the samples for 6-hour sequencing, and some for 48-hour sequencing,

but in every experiment, we either got the same amount of data or more inflight than we did on the ground, and the accuracies were all slightly higher inflight than on the ground. Without being able to get the flow cells back and take them apart and really try to understand what's going on, we noticed it's a little bit better, but it's really hard to come-up with a good explanation why.

Man 1: Is there a plan that you try to get some of the flow cells back?

Dr. Aaron Burton: Yes. We've gotten some of them back. The problem is they have liquid in them and then they dry out. So they kind of just get all crusty and sort of hard to work with.

Man 1: Changed after the flight.

Dr. Aaron Burton: Yes. There could be some more in-depth stuff; we can do to look at the flow cell quality. Just in the metrics of the data that we got to see if there's any sort of trend there; that's a goodquestion.

All right. We'll move on to the next slide [Slide 20].

We saw no decrease in sequencing performance on ISS compared to on the ground. We got 284 thousand reads on the ISS, which was about 50% to 70% more than we got on the ground.

This data we were able to basically reconstruct the entire E. coli genome, so that's -- I think -- around 2.6, 2.7 million bases. We also did the bacteria -- excuse me -- the virus, the bacteriophage lambda. That wasn't very hard because it was only 50,000 bases. The mouse genome was much, much bigger. It's about three orders of magnitude bigger than E. coli, so we

weren't able to reconstruct that, but we could do the mitochondria. The genome of that, which is the little power plant of the cell.

Then we were also able to do de novo genome assembly of the virus and the bacteria. Basically, if you had no idea what was in your sample from the reads that we got, you could put together the entire genome, which was a pretty impressive accomplishment. It was way better than we'd hoped to get.

Then lastly, we demonstrated that the flow cells could be reused, and they had shelf-life stability out to at least six months in space.

The figures on the right just show the genome assemblies and coverage.

On the next slide [Slide 21], we went into this and we sent up samples that we had prepared on the ground, but it's not a very cost effective science approach to prepare samples on the ground and then fly them to the ISS to be analyzed. We wanted to be able to prepare the samples for sequencing on the ISS. So we developed a method and we had astronauts and other crew members test them out on NEEMO, which is NASA's underwater analog. This process looks like swabbing a surface, extracting the DNA, amplifying it when there's not very much, and then getting the DNA ready for sequencing, and then doing the analysis. And so we tested this at NEEMO.

If you go to the next slide [Slide 22], here are pictures of the NEEMO crew members doing bacterial swabs, the DNA extraction; this is meanwhile on the ocean floor. Mini-PCR amplification, sample preparation, loading the MiniION, and then successful sequencing.

So it was really exciting when Reid Wiseman was able to go through this entire process because we had had Kate Rubins who actually came to our lab before she flew up to the ISS, and actually do the experiments on the ground. To be able to have someone that had never seen the hardware before do it was really great.

The next slide [Slide 23].

Here's just a quick comparison of a molecular biology lab on the ocean floor.

[Slide 23]. So here's the samples that were brought back from the NEEMO experiment; we analyzed them on the aluminum IC, which is kind of the gold standard instrument. Then the Oxford nanopore technologies MinION. We compared the species that were identified and they were a pretty good match. So we were satisfied with that.

I'm trying to just finish-up so I don't run over on time.

So if we go to the next slide [Slide 24], Genes in Space 3 are some more recent experiments that we did that we're very excited about.

And so the ultimate goal of this experiment was to be able to take a sample that we collect on the ISS, and use that procedure that we tested at NEEMO to actually be able to identify microorganisms. The overall workflow for this -- if you look at the picture – is taking a growth plate that we already do on the ISS. Collecting cells from that, and being able to extract the DNA and amplify a diagnostic gene. Then do the sequencing library preparation, and then actually do the sequencing on the ISS without having to rely on anything being returned to Earth to do the identification.

So if we go to the next slide, which is [Slide 25]...

So here is Peggy Whitson actually getting ready to start doing this process.

And if we go to the next slide [Slide 26]...

So, the first thing we did was use DNA that we had sent up that was purified just to show that all the pipetting steps work. This is all done with just standard Eppendorf pipetters that you would use in your lab on Earth. We didn't engineer, or really build anything, we just decided to test it out and see if it worked.

You could see the two plots are events, which is a proxy for a sort of a number of molecules sequenced. Then read length histograms – and so, again, an as identical of experiments as we could do, the ISS one has 328,000 reads - and I apologize that that font is really small, and the ground had 260,000. We got about 25% more reads on the ISS than we did on the ground.

Everything worked really well. So then we decided to try the homerun experiment, which is next slide [Slide 27] – the Genes in Space 3 Med Ops Sample.

Here is Peggy actually collecting cells from this growth media slide; just picking it directly from the colonies, and you can put that right into a PCR. The heat from the PCR burst the cells open, and then you also amplify the 16s gene, which all bacteria have, but it contains enough variations that you can actually identify organisms based on those variations.

And so this process all worked really smoothly.

So if we go to the next slide – [Slide 28].

I apologize because this was a medical operations slide, all the international partners have to basically get all the data before it can go public. So I can't tell you what she identified in there, but we did the sequencing in space and got that data back, and then we got that actual sample back and processed it through our standard procedures on the ground to validate it.

Those three colonies were 100% match at the species level with what we identified on the ground. So that was a huge – sort of a huge, exciting moment for us.

Let's go to the next slide, [Slide 29].

Dr. Aaron Burton: So we identified with just stuff that we did inflight what those organisms were. And then we were able to confirm it on the ground.

And so with this success in hand, we've been working to get established as a facility for researchers to use on the ISS. We've got a whole list of reagents that are certified for flight to make it easy for researchers; consumables, so just standard lab equipment that you would use. Now researchers won't have to go through all the work of certifying those; and then we have the crew procedures already developed so that, hopefully, researchers will be able to use this.

Man 2: Dr. Burton, can you fly enough MinION flow cells to demonstrate that you can extend the shelf life beyond the six months that you've got right now?

Dr. Aaron Burton: Yes. So we're working on that. And a lot of that is just, yes, fly them up there, keep them in the fridge, and then have somebody test them. Yes.

We have some that flew in April that are still up there, and haven't been used. So we're at six months now; hopefully we can get some crew-time because it only takes a half hour depending on the experiment to actually just run those and make sure that they're still good.

That's a very good question.

And so slide [Slide 30], is just types of questions that we would want -- or that people might want -- to answer with their ISS research and researchers are smarter than we are, so we had just tried to make the platform as flexible as possible so that they could do whatever they wanted to do.

Then on [Slide 31], are acknowledgments. We've had a lot of help doing this and everybody's played a pretty important role. In the upper left hand corner is Sarah Wallace, Sarah Stahl, myself, and Kristen John, who are kind of the Core 4 at JSC, that did a lot of the paperwork; Sarah Stahl did a lot of the method development.

In the center, is our kind of expanded science team, which has team members from Goddard, Ames, as well as a couple of universities – Weill Cornell Medical College, and University of California at San Francisco -- have helped us with data analysis.

The company Oxford Nanopore Technologies has been really excited about this and very supportive. Genes in Space 3 – so Scott Copeland and the mini-PCR team – Zeke and Sebastian were really helpful.

So it's been a lot of help.

And if we go to the last slide, just some fun tweets.

Heather Doyle: Great. Thank you so much.

Dr. Aaron Burton: Yes.

Heather Doyle: And one question I have.

Dr. Aaron Burton: Thank you all.

Heather Doyle: Yes. This is great and I appreciate all the nuances to it including right now you have humans transferring the sample the PCR and the MinION, right. So is there [someone] looking at maybe trying to do it with robots or is this something a robot could handle on its own without human intervention at some time in the future or...

Dr. Aaron Burton: Yes. So I think in principle it definitely should be. Oxford is developing a sample prep platform that you would just basically pipette your extracted DNA into and then pull out the liquid in the end that would go directly into the MinION. And so you could picture just having a robot do that.

So we did all of this. We started this project with, like, money and a flight in February of 2015. And so we've been just- like make it work. It is exactly as it is now. Get something out of it. And so certainly looking at automating it would be great.

Heather Doyle: Wonderful.

Anybody else have any other questions out there?

Jeff Nee: So no plans to go on the Mars 2020 Rover for example, right?

Dr. Aaron Burton: Well, I would love that, but I think that payload was pretty much set in 2014. I would love to get on there, but I think we need to be, you know, three or four TRL levels higher before we could be at a real candidate.

Man 4: Hi. I had a few questions actually.

Regarding [Slide 14] where the mini-PCR and the QPCR was done...

Dr. Aaron Burton: Yes.

Man 4: ...was that isolated on the ISS and what organisms were used?

Dr. Aaron Burton: Let's see. So that was... I know that they flew a cell line – if you want to send me an email, I can email you the paper.

Man 4: Okay.

Dr. Aaron Burton: Yes. Because that one's published in PLOS 1.

Man 4: Okay, I actually I had another question. I know you mentioned that you guys didn't have to engineer anything special, like pipetters or Effendorf tubes. Were there any issues when actually working with the samples? I mean working with microliter amounts, so were there any issues with the samples floating away or anything like that?

Dr. Aaron Burton:

Yes, so pipetting on Earth you pipette and the liquid comes-up to where it comes-up in the pipette tip. But in microgravity, it continues to creep up the side because it doesn't have gravity kind of pushing it down. And so you have to be reasonably quick to do that or the positive displacement pipettes. It's effectively like a syringe in the tip and so you don't have that issue, but they're a little harder to work with.

Man 4: Okay.

Dr. Aaron Burton: But that worked-out okay.

So the PCR tubes are pretty small. They're, like, 200-microliter tubes. And so sometimes if you got an air bubble in the bottom of those, it could be hard to remove. So normally you would just centrifuge it, but we didn't have that, so there was a lot of kind of shaking the tube. You hold the top of it and kind of shake it around as hard as you can.

Dr. Aaron Burton: Yes. If it's a big enough one, you can envision just pipetting out the air so that the liquid kind of gets sucked-down in there.

But overall it went as good as we could've hoped.

Man 4: Awesome. Cool. Thank you.

Man 5: Dr. Burton, you mentioned reaching-out to you through email. What is your email? How would we reach you?

Dr. Aaron Burton: Sorry. I should have put that in here. It's Aaron.Burton@NASA.gov

Jeff Nee: I just have a question. Petty early on I like how you referred to prebiotic as kind of a magical time, where we're not really sure what's going-on. How... I know there are labs out there that are trying to simulate that prebiotic time. Do we have a sense of how close are we to getting some sort of answer? Like, for example, some people say that artificial intelligent life is, you know, 50 to 100 years away. Do you think we'll see an answer to how that prebiotic time evolved into that RNA world within, say, our lifetimes?

Dr. Aaron Burton: Yes. That's a very good question. And people in the prebiotic chemistry community fight about it. From a philosophical standpoint a lot of people say that we'll never know exactly how life started on Earth. And the best that we can hope to do is sort of recreate that. So find the chemical reactions that work and maybe eventually you could build a network of molecules that's able to reproduce and then turn into some things that we would recognize as a alive.

In some sense, that would almost be like synthetic biology, right. You kind of build your life, but you wouldn't know that that's exactly how life on Earth started.

Does that make sense?

Jeff Nee: Yes. So I guess my question is how close are we to synthetic biology being a reality? Fifty years, 100 years, 1,000 years, I mean, just to throw-out a number
– in your opinion of course.

Dr. Aaron Burton: Yes. I could see in the next 100 years. We're learning a lot about how genomes are put together and the James Craig Venter Institute did an experiment where they basically took a bacterial genome and they chopped into pieces, rearranged it all, and then put it back together, and then put it into a cell that had proteins and had everything in it except DNA. That

organism was able to live. And then people have done things where they entirely change-out amino acids and get them to work.

So, I think we're not that, I mean, in terms of our lifetimes maybe we are that far away. But probably in the next 100 years it doesn't seem that farfetched.

Jeff Nee: Thanks. And then I have just one final question. So you mentioned it's important for the nanopore to be able to detect multiple versions of DNA and RNA alternatives. With the signals, you gave us a couple of graphs. I'm just curious if the signals would be noticeably different if it was not DNA or RNA, or if it kind of looks the same shape -- at least -- for the graph.

Dr. Aaron Burton: Yes. So that's going to be the challenge in that the closer it is to DNA or RNA...

So there's a group at University of California at Santa Cruz - so Mark Akeson - and they have some work that's going to come-out soon where they've been looking at ribosomal RNAs, and those can be pretty extensively modified. And so they're able to detect single atom substitutions or changes in those -- so hydrogen for, like, a methyl group, or that sort of thing.

So in principal, yes. I think as you get farther away from what we recognize and know how to work with, I think it will be harder

For me there's kind of philosophical thing that if you were on the surface of Mars, and you found something that was a poly sort of electrolyte, but it didn't look like DNA or RNA, that would be a huge amazing and exciting thing and I would really want to get those samples back on Earth to look with more precise tools.

But yes, I mean, I think the potential is there.

Jeff Nee: Great. Thanks. I love that cartoon you put on the -- what is it – [Slide 13], the one in the corner. I just think that's great. So thanks.

Heather Doyle: All right. Well if there aren't any other questions, I just want to thank you again, Dr. Burton, for your time. And thank you everyone who called-in. It was a really interesting and cutting-edge topic. So, it was great to hear from you about it.

Man 1: Definitely. One thing I would add is when can we get Dr. Burton back to talk about his other areas of research, including determining the origin of organics and meteorites?

Heather Doyle: We'll follow-up on that one for you.

Do remember that this talk is archived at NASA Nationwide and the Museum Alliance site, so you can share this presentation anytime in the future.

The next one we have coming-up is actually tomorrow. We have Universe of Learning: Multi-messenger Astronomy, A New Era in Space Science tomorrow at 12:30 Pacific. Jeff Nee will be leading that one.

So if you think of any other questions or need anything else, feel free to email me at hdoyle@jpl.nasa.gov. But if that's it then we will speak to you tomorrow.

Thank you everybody.

END